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Effects of morphine on catecholamine release and arrhythmias evoked by myocardial ischaemia in rats

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1 The effects of morphine ($10\,mg\,kg^{-1}$ i.p.) on haemodynamics, arrhythmias and plasma and myocardial catecholamines (CA) were studied after coronary artery occlusion in anaesthetized rats. Myocardial intraneuronal CA were assessed histofluorimetrically and CA concentrations measured by high performance liquid chromatography.

2 Morphine increased blood pressure, presumably due to higher plasma noradrenaline (NA) concentrations found in morphine-treated rats.

3 Morphine increased the area of catecholamine-containing fluorescing neurones in the myocardium (as a percentage of total field area) 60 min after sham-operation ($0.87 \pm 0.07\%$) or occlusion ($0.57 \pm 0.05\%$) compared to untreated animals (0.67 ± 0.06 and $0.38 \pm 0.03\%$ respectively). Tissue NA content was not significantly affected by coronary occlusion and/or morphine within the first 60 min.

4 Morphine had no effect on ischaemia-induced arrhythmias.

5 Whether the higher intraneuronal NA content following morphine resulted from reduced central sympathetic outflow to the heart, presynaptic inhibition of NA release, or increased uptake due to higher plasma concentrations is unclear. Ischaemia-induced local NA release appears independent of these mechanisms, as it was unaffected by morphine.

Introduction

In the early period of myocardial infarction, the occurrence of ventricular arrhythmias (VES) and ventricular fibrillation (VF) is the main cause of early, prehospital mortality. About 60% of deaths occur during the first 2 h after the onset of symptoms (Kannel & Thomas, 1982).

In addition to biochemical, metabolic and mechanical factors (Opie *et al*, 1979; Covell *et al*, 1981), the effects of catecholamines (CA) on the ischaemic and non-ischaemic myocardium seem to play an important role in the genesis of early arrhythmias. In several studies, a positive correlation between adrenergic activity and the incidence of arrhythmias has been demonstrated (Lown & Verrier, 1976; Sharma & Corr, 1983). Two different mechanisms seem to be responsible for the increased concentration of CA at the myocytes: (1) Due to pain, fear and haemodynamic alterations, the activity of the sympathetic nervous system is increased by central reflexes (Kliks *et al.*, 1975). (2) A reflex-independent local release of noradrenaline (NA) in the ischaemic myocardium, probably due to hypoxia, acidosis and increased extracellular potassium concentration has been described by several investigators (Holmgren *et al.*, 1981; Abrahamsson *et al.*, 1982; Hirche *et al.*, 1985).

Morphine is frequently used as an analgesic in patients suffering from myocardial infarction because of its rapid and reliable onset of analgesia, sedation of the anxious patient and its almost negligible effects on myocardial contractility and haemodynamics (Jaffe & Martin, 1980). Opiates have been shown to inhibit NA release from sympathetic nerve terminals by central sympatholysis (Laubie *et al.*, 1977) and by interaction with inhibitory presynaptic opiate binding sites on adrenergic nerve terminals in a variety of organs (Henderson *et al.*, 1979; Stickney & Eickenburg, 1981).

It was the purpose of this study to investigate the effects of morphine ($10\,mg\,kg^{-1}$, i.p.) on systemic and local CA release in the ischaemic myocardium and the incidence of early arrhythmias after coronary artery occlusion in the rat.

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Methods

Experiments were performed on male rats (Ham Wistar) weighing 250–350 g. Anaesthesia was induced with pentobarbitone sodium (6 mg 100 g^{-1}, i.p.). The animals were ventilated artificially with room air (1 ml 100 g^{-1}, 60–70 strokes per min) with a Rodent Respirator 681A (Harvard App., Mass., U.S.A.) and set up to allow recording of arterial blood pressure, from the left common carotid artery, (Statham P23DB, Hato Rey, Puerto Rico) and the electrocardiogram (ECG) using standard limb leads. These parameters were recorded continuously on a four-channel recorder (Gould Brush 2400).

Rectal temperature was maintained at approximately 38°C. After left thoracotomy in the fifth intercostal space, sectioning of the fourth and fifth ribs approximately 2 mm from the left margin of the sternum and resection of the pericardium, the heart was exteriorised by gentle pressure on the right chest wall. A ligature (6–0, 0.7 metric Perma-Hand Seide, Ethicon) was placed around the left coronary artery close to its origin and the heart was replaced immediately in the chest cavity.

Experimental design: rats were divided into four groups (Table 1). Immediately after placing the ligature, NaCl 0.9% 1 ml kg^{-1} (groups I and II) or morphine HCl 10 mg kg^{-1} (groups III and IV) was administered intraperitoneally. This dosage has been shown to produce significant analgesia in rats for 1 h after administration (Clarke & Wright, 1984). After 15 min, the left coronary artery was occluded in groups II and IV.

In 5 rats of each group, 15 min after sham-operation or occlusion (i.e. 30 min after morphine or NaCl administration) 3 ml of arterial blood was taken from the carotid artery for the measurement of plasma CA concentrations. These animals were then excluded from further experiments.

In the remaining animals the hearts were rapidly removed and shock-frozen, by means of a Wollenberger clamp cooled in liquid nitrogen, 60 min after sham-operation or occlusion. The hearts were stored at −80°C until analysis. Frozen tissue samples were taken from the left ventricular anterior wall, representing ischaemic myocardium in rats with coronary occlusion, and non-ischaemic myocardium in sham-operated rats. Fluorescence of intraneuronal catecholamines was induced by the method of De la Torre (1980) in cryosections which were 16 μm thick and cut 200 μm subepicardially. The area of fluorescing adrenergic nerve fibres was assessed morphometrically by means of a Leitz u.v. microscope coupled to a residual light amplifying caesicon video camera (PIC 762, Kranz, F.R.G.) and a picture analysing system (Artec Counter 982, Fisher Sci., F.R.G.). The area of fluorescing fibres was expressed as a percentage of the total field area in 100 microscope fields (total area 3 mm^2) per section. In the remaining tissue sample and in plasma, CA were determined by high performance liquid chromatography (h.p.l.c.) using electrochemical detection (h.p.l.c. catecholamine analysing system, Waters GmbH, FRG).

Dysrhythmic activity was assessed from ECG and blood pressure recordings. The number of ectopic beats per minute was counted. A run of 7 or more consecutive ectopic beats at a higher than normal rate was defined as ventricular tachycardia (VT). Periods of irregular, high frequency electrical activity combined with a fall in blood pressure to almost zero were defined as ventricular fibrillation (VF). VT and VF were expressed as seconds per minute.

Statistical evaluation of the data was performed using Student's t test or the Chi-squared test. The 5% level was accepted as significant. All values are expressed as mean ± standard error (x ± s.e.mean).

Table 1 Number of animals (n) in each experimental group

	Morphine			
Group I	0	Sham-op	Plasma CA (5)	Myocardial CA (10)
Group II	0	Occlusion	Plasma CA (5)	Myocardial CA (12)
Group III	10 mg kg^{-1}	Sham-op	Plasma CA (5)	Myocardial CA (10)
Group IV	10 mg kg^{-1}	Occlusion	Plasma CA (5)	Myocardial CA (12)
	−15	0	15	60 min

CA = catecholamines

Results

Haemodynamics

Figures 1 and 2 show the changes in mean arterial blood pressure (MAP) and heart rate (HR) in animals used for the assessment of myocardial CA. Thoracotomy and artificial ventilation caused a significant fall in MAP of about 30% ($P < 0.01$) in all animals to values between 90 and 120 mmHg (see Figure 1). In the first 15 min following the administration of morphine or NaCl, there were only slight changes in MAP in animals receiving NaCl, whereas the morphine-treated rats showed a significant increase in MAP ($P < 0.001$). In group III (sham-operated), this increase continued until 30 min after morphine administration to reach a maximum of 160 mmHg, followed by a slow decrease until the end of the experimental period. Coronary artery occlusion (groups II and IV) caused a fall in MAP in the first minute to values of 76 ± 6 mmHg (group II) and 82 ± 5 mmHg (group IV; both $P < 0.01$). In group II, MAP remained significantly decreased compared to pre-occlusion values until 30 min after the onset of ischaemia. In the morphine-treated animals, the depression of blood pressure lasted only until 5 min after occlusion. After 15 min, MAP was significantly elevated above pre-occlusion values in the morphine-

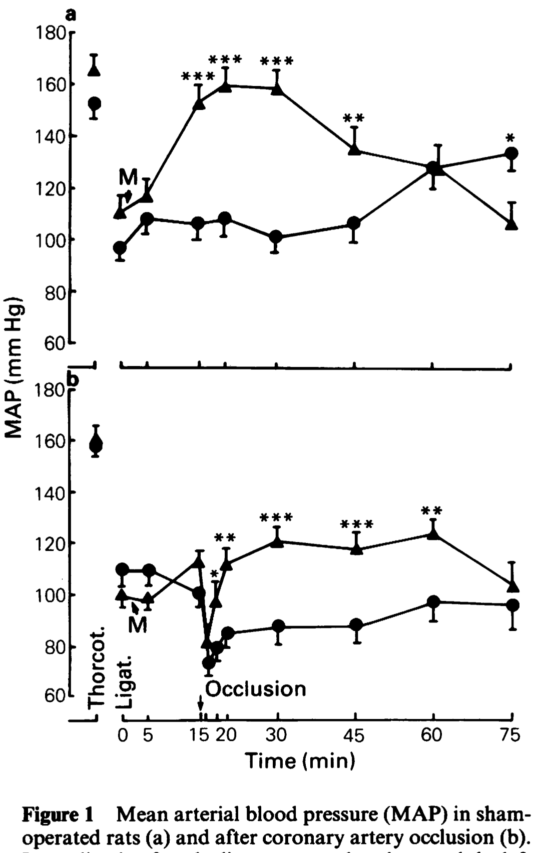

Figure 1 Mean arterial blood pressure (MAP) in sham-operated rats (a) and after coronary artery occlusion (b). Immediately after the ligature was placed around the left coronary artery, the animals received morphine hydrochloride 10 mg kg^{-1} (M, ▲), NaCl 0.9% 1 ml kg^{-1} i.p. (control ●). In (a) $n = 10$ for morphine-treated and control rats; in (b) $n = 12$ for morphine-treated and control rats. Points are mean values with vertical lines indicating s.e.mean. *$P < 0.05$; **$P < 0.02$, ***$P < 0.001$ compared to untreated animals.

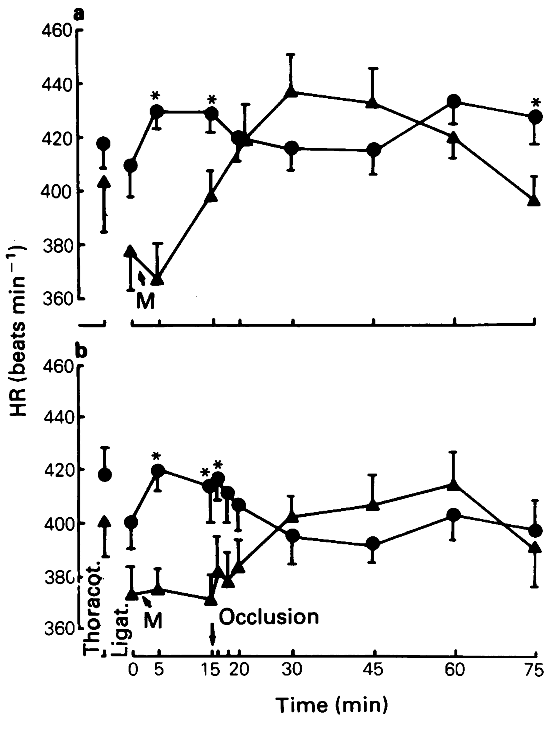

Figure 2 Heart rate (HR) in sham-operated rats (a) and after coronary artery occlusion (b). Immediately after the ligature was placed around the left coronary artery, the animals received morphine hydrochloride 10 mg kg^{-1} (M, ▲) or NaCl 0.9% 1 ml kg^{-1}, i.p. (control, ●). In (a) $n = 10$ for morphine-treated and control animals; in (b) $n = 12$ for morphine-treated and control animals. Points are mean values with vertical lines indicating s.e.mean. *$P < 0.05$ compared to untreated animals.

Table 2 Mean arterial blood pressure (MAP) and heart rate (HR) after sham-operation (groups I and III) or coronary artery occlusion (groups II and IV)

				Minutes after placing the ligature		
		Pre-Thorac.	*Pre-lig*	5	15 (Pre-occl.)	30
Group I	MAP	144 ± 9	116 ± 8[a]	109 ± 7	142 ± 14	129 ± 15
	HR	350 ± 23	360 ± 23	361 ± 34	368 ± 34	353 ± 37
Group II	MAP	162 ± 10	96 ± 7[aa]	83 ± 12	87 ± 11	76 ± 6
	HR	398 ± 18	360 ± 14[aa]	395 ± 17	368 ± 11	355 ± 9
Group III	MAP	148 ± 14	114 ± 12	104 ± 9	172 ± 5[bb]	180 ± 11[bb]
	HR	356 ± 21	346 ± 15	294 ± 12[b]	389 ± 16[b]	437 ± 18[bb]
Group IV	MAP	168 ± 11	96 ± 7[aa]	103 ± 15	130 ± 20	145 ± 5
	HR	400 ± 18	377 ± 8	349 ± 7[b]	365 ± 25	415 ± 19[c]

Immediately after placing the ligature, the rats received 0.9% NaCl 1 ml kg^{-1} (groups I and II) or morphine hydrochloride 10 mg kg^{-1}, i.p. (groups III and IV); 30 min later, 3 ml of arterial blood was taken for the determination of plasma catecholamine concentrations.
$\bar{x}$ ± s.e.mean, $n = 5$, [a]: $P < 0.05$, [aa]: $P < 0.01$ compared to pre-thoracotomy; [b]: $P < 0.05$, [bb]: $P < 0.01$ compared to pre-ligature; [c]: $P < 0.05$ compared to pre-occlusion.

treated rats. Morphine-treated rats also showed significantly higher MAP after both sham-op (group III) or coronary occlusion (group IV) compared to untreated animals (groups I and II respectively, Figure 1).

The changes in HR seen were less pronounced. In morphine-treated rats, HR was significantly lower for the first 15–18 min after administration compared to animals receiving NaCl (Figure 2).

Table 2 shows the haemodynamic changes in animals used for the determination of plasma CA. As can be seen, the haemodynamic changes were similar to those observed in animals used for the assessment of myocardial CA.

Arrhythmias

After coronary artery ligation, all animals developed arrhythmias in the first 30 min of ischaemia. The severity of these arrhythmias is shown in Table 3. Morphine had no significant effect on the arrhythmias developing as a result of coronary occlusion.

Catecholamines

Plasma CA concentrations were determined 30 min after NaCl or morphine administration (i.e. 15 min after sham-operation or occlusion). In a separate group of 5 rats, blood samples were taken immediately after thoracotomy to serve as control. These values are shown in Table 4. Thirty minutes after NaCl, significantly ($P < 0.01$) higher adrenaline (Ad) concentrations were found in sham-operated rats compared to control. In morphine-treated rats, plasma NA concentrations were almost three times higher than in untreated animals, and the increase in plasma Ad was not seen after morphine pretreatment.

Sixty minutes after coronary artery occlusion, the fluorescing area in the ischaemic myocardium was reduced by 49% compared to sham-operated hearts ($P < 0.001$, Figure 3). After morphine treatment, both ischaemic and non-ischaemic hearts showed a significantly higher fluorescing area than untreated hearts. However, the reduction in fluorescence of 35% at 60 min compared to morphine-treated, sham-operated hearts was almost the same as in untreated animals (Figure 3).

NA tissue concentration in the left ventricle was reduced by 14% after 60 min of ischaemia in untreated rats and by 13% after 60 min of ischaemia in morphine-treated rats compared to the appropriate sham-operated group. Morphine-treated rats showed slightly higher myocardial NA content after sham-operation or occlusion than untreated rats, however, none of these differences was statistically significant (see Figure 3).

Table 3 Ventricular arrhythmias following left coronary artery occlusion in untreated and morphine-treated rats

	Untreated	*Morphine-treated*
Number of rats	12	12
VES per 30 min	614 ± 166	618 ± 174
Number of rats with VT	11 (92%)	10 (83%)
Mean duration of VT (s)	27 ± 8	11 ± 2
Number of rats with VF	2 (17%)	5 (42%)
Mean duration of VF (s)	47 ± 2	11 ± 5

$\bar{x}$ ± s.e.mean, VES: ventricular extrasystoles; VT: ventricular tachycardia; VF: ventricular fibrillation.

Table 4 Plasma concentrations of noradrenaline (NA) and adrenaline (Ad) immediately after thoracotomy (Control) and 30 min after administration of 0.9% NaCl ($1\,ml\,kg^{-1}$) or morphine hydrochloride ($10\,mg\,kg^{-1}$) and sham-operation or coronary artery occlusion

		Sham-operated		*Occlusion*	
	Control	30 min + NaCl	30 min + morphine	30 min + NaCl	30 min + morphine
NA ($pg\,ml^{-1}$)	138 ± 18	135 ± 51	363 ± 54*	92 ± 12	306 ± 93
Ad ($pg\,ml^{-1}$)	214 ± 38	423 ± 42	225 ± 80	563 ± 128	81 ± 61*

x ± s.e.mean, $n = 5$, *: $P < 0.02$ compared to the values obtained after NaCl.

Discussion

Haemodynamics and arrhythmias

In untreated rats, coronary artery occlusion led to a reduction in MAP with no significant changes in HR. Similar haemodynamic effects have been described by other investigators (Clark *et al.*, 1980; Au *et al.*, 1983) and are presumably a consequence of reduced stroke volume due to alterations of ventricular function caused by ischaemia (Pfeffer *et al.*, 1979).

Ventricular ectopic activity began soon after occlusion and lasted approximately 30 min, with a maximum rate of 102 ± 48 VES min^{-1} after 8 min. Ventricular fibrillation or tachycardia were seen only during the first 15 min. These observations are also in agreement with those of other investigators using the same model (Clark *et al.*, 1980; Marshall *et al.*, 1981; Au *et al.*, 1983; Campbell & Parratt, 1983). Our finding of a total of 614 ± 166 VES during the first 30 min of ischaemia is lower than that reported by some other groups of workers. This may be due to differences in the strain of rats used (McCarty *et al.*, 1979), differences in the amount of stress to which the animals were exposed (the animals used in this study were bred in our Institute, and therefore not exposed to transport or cold stress), or periodic changes in the sensitivity of rats to arrhythmogenic factors (Abrahamsson & Almgren, 1981).

Catecholamines

An increased release of catecholamines in the ischaemic myocardium has been found in a number of species, such as the dog (Mathes & Gudbjarnason, 1971), pig (Hirche *et al.*, 1985; Holmgren *et al.*, 1985), and the rat (Abrahamsson *et al.*, 1982). However, the extent and time course of NA release and its underlying mechanisms are not yet completely clear.

We investigated myocardial catecholamines using two different methods. The morphometric assessment of fluorescing adrenergic nerve terminals in relation to

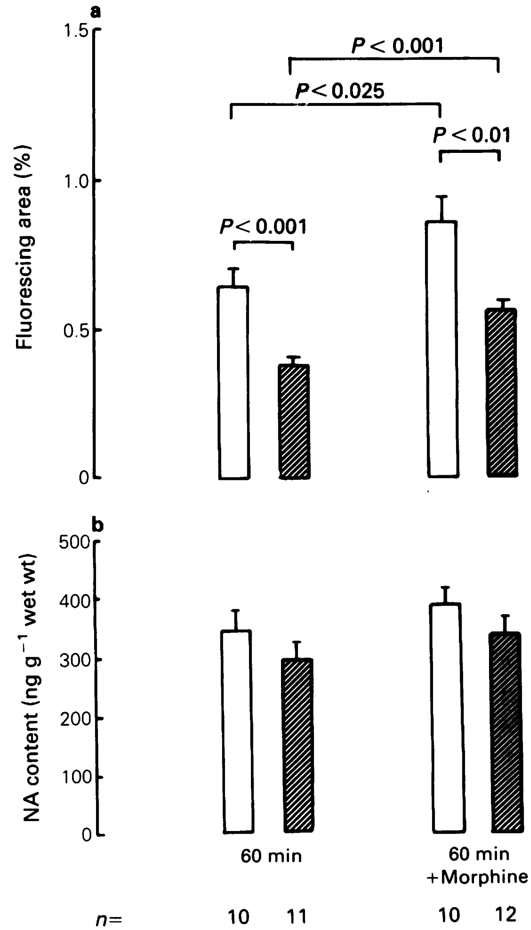

Figure 3 Fluorescing nerve fibres (as percentage of total field area) (a) and noradrenaline (NA) content ($ng^{-1}\,g$ wet wt) (b) in samples of left ventricular myocardium 60 min after sham-operation (open columns) or coronary artery occlusion (hatched columns) in untreated and morphine-treated rats. Columns show mean values with vertical lines indicating s.e.mean.

total field area reflects the intra-neuronal NA content within certain limits. Jonsson (1971) showed in the rat iris that neurones which still contain 5–10% of their normal NA content no longer show a visible fluorescence, and that an increase above 40% of the maximum NA content is not accompanied by a further increase in fluorescence. This may explain why the perivascular sympathetic nerve plexuses preserve a visible fluorescence longer than terminal fibres in the ischaemic myocardium (Paessens & Borchard, 1980). For morphometric assessment we therefore excluded areas which contained such sympathetic plexuses. The determination of total tissue NA content measures both intra- and extra- neuronal NA, and this may explain the relatively poor correlation we found between values of fluorescing area and NA content ($r = 0.664$, $n = 28$, $P < 0.001$).

In agreement with Abrahamsson *et al.*, (1982) and Holmgren *et al.*, (1981), we found a significant reduction in fluorescing nerve fibres in the ischaemic myocardium after 60 min of occlusion compared to sham-operated animals. NA tissue content at this time was reduced by only 14%. The difference between the marked reduction in intra-neuronal CA and the only slight decrease in total NA content implies high extraneuronal CA concentrations. This may be a consequence of a decreased inactivation of released NA in the ischaemic myocardium. Reuptake, the most important mechanism of inactivation under normal conditions, is reduced by ischaemia in the rat heart (Schömig *et al.*, 1982). Washout of released NA, and the activity of the catabolic enzymes monoamine oxidase (MAO) and catechol-*O*-methyltransferase (COMT) would also be expected to be reduced under conditions of reduced blood flow and hypoxia.

Morphine effects

In clinical studies, reductions in blood pressure and vascular resistance after morphine administration have been described (Sethna *et al.*, 1982) but also hypertonic (Conahan *et al.*, 1973; Kistner *et al.*, 1979) or unchanged (Lee *et al.*, 1976) haemodynamics. Animal experiments have also shown differing haemodynamic effects, depending on route of administration, dose, and the additional effects of anaesthetics (Fennessy & Rattray, 1971; Holaday, 1983). In our experiments, morphine administration led to significant increases in MAP. This is presumably a result of the higher plasma NA concentrations found 30 min after morphine administration, when the haemodynamic effects were maximal. In preliminary studies, we found that a lower dose of morphine ($5\,\mathrm{mg\,kg^{-1}}$, i.p.) had no marked effects on either plasma CA or haemodynamics. Fennessy & Rattray (1971) showed that the increase in MAP seen in the rat after morphine could not be prevented by adrenalectomy or hexamethonium, but was blocked by the α-adrenoceptor antagonist, phentolamine. They concluded therefore that, in the rat, morphine causes NA release from peripheral sympathetic nerves. Increased NA concentrations in plasma from rats treated with morphine have also been described by other investigators (Van Loon *et al.*, 1981; Conway *et al.*, 1983).

In the myocardium of morphine-treated rats 60 min after sham-operation or occlusion, a slightly higher NA content and a significantly higher area of fluorescing neurones was found than in untreated rats. This increase in fluorescing area suggests a higher intraneuronal NA content, and may be due to a number of possible mechanisms: (1) A morphine-induced 'central sympatholysis' (Laubie *et al.*, 1977) could reduce sympathetic outflow to the heart and so diminish NA release. This effect, however, does not seem to be present in all sympathetically-innervated organs, as the increased plasma NA concentration shows. (2) The higher intraneuronal NA content may be a consequence of an increased uptake of NA resulting from the higher plasma NA concentration. The heart has been shown to be capable of taking up about 80% of circulating CA during a single passage (Wurtmann *et al.*, 1963). (3) The increase in plasma NA and decrease in plasma Ad concentrations may reduce NA release via presynaptic α_2- or β-adrenoceptors. (4) Morphine may reduce NA release by presynaptic opiate receptor sites. The results reported for myocardial preparations are, however, controversial. In the rabbit heart, Starke (1977) found an increased NA release after morphine, whereas in guinea-pig isolated atria, a dose-dependent reduction in [^{3}H]-NA release has been shown for etorphine (Fuder, 1985). (5) Morphine may lead to an increased synthesis of CA. Increased CA concentrations as well as increased activity of tyrosine hydroxylase and dopamine-β-hydroxylase have been found in brain, adrenal medulla and plasma after morphine treatment (Anderson & Slotkin, 1976; Prasad *et al.*, 1976).

Which of these possible mechanisms plays the most important role in the increase in myocardial intraneuronal NA after morphine treatment found in the present study cannot, however, be determined from the available data.

The reduction in intraneuronal fluorescence in the ischaemic myocardium was not affected by morphine. This local release seems to be independent of efferent sympathetic impulses (Dart *et al.*, 1984). It appears to be mediated by a calcium-independent carrier, possibly identical to the uptake carrier, and ischaemia-induced increases in membrane permeability may also be involved (Schömig *et al.*, 1984). It has previously been shown that morphine can reduce nicotinic-induced, but not KCl-induced NA release from chromaffin cells (Kumakura *et al.*, 1980). In contrast to meptazinol, a partial agonist at opiate receptors which has been shown to have antiarrhythmic properties (Fagbemi *et al.*, 1983), in our experiments morphine also had no effect on arrhythmias.

In conclusion, morphine administration increased plasma NA concentration and the NA content of adrenergic nerves in the rat heart. It is not possible on the basis of the present results to determine whether this was primarily a consequence of increased uptake of NA as a result of the increased plasma concentration, or whether morphine directly inhibited NA release, possibly via presynaptic opiate receptors. The local release of NA in the ischaemic myocardium was unaffected by morphine, as were the early ischaemia-induced arrhythmias, which may suggest a causal role for locally-released catecholamines in the genesis of these arrhythmias in the rat.

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